The Effect of Host Food Quality on Host and Parasite Fitness in an Invertebrate-Parasite System

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The Effect of Host Food Quality on Host and Parasite Fitness in an Invertebrate-Parasite System

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SUMMARY

Parasites achieve their fitness by reducing the fitness of the hosts they infect. The relationship between host and parasite fitness is often mediated by environmental conditions, such as the quality of food consumed by the host. We used the crustacean *Daphnia dentifera* and its virulent yeast parasite *Metschnikowia bicuspidata* to examine how the quality of the food consumed by the *Daphnia* affected the probability of the host being infected by the parasite and the fitness consequences of infection for the host (measured as offspring production and survival) and for the parasite (measured as the production of transmission spores within the host following infection). We fed *Daphnia* either high quality food (*Ankistrodesmus falcatus*) or low quality food (*Oocystis sp.*) before exposure to *Metschnikowia* spores, and then either high quality or low quality food after parasite exposure (according to a fully factorial design). We found that when hosts were fed high quality food as juveniles and adults, they were able to invest more energy in preventing loss of fitness due to parasites without limiting parasite growth; high quality food therefore benefits both parasite and host. High food quality benefited both host and parasite when hosts are infected. We saw that infected hosts that produced more offspring also contained more parasite spores. One possible explanation for this is the individuals that produced more offspring and spores were larger, allowing them to take in more resources.

CHAPTER 1

INTRODUCTION

Parasitism is a common interaction in which the parasite gains fitness at the expense of the host. Parasites apply considerable selection on host populations (Hall *et al*. 2011; Duffy *et al*. 2012; Thrall *et al*. 2012), and this selection can manifest in a variety of ways. Host immune defense is often costly to maintain, so investment in immune defenses can constrain investment in other fitness-related traits (Kraaijeveld & Godfray 1997; Sheldon & Verhulst 1996). Parasites often select for host resistance, i.e. the capacity to either prevent infection from occurring or to eliminate the infectious agents with an immune response. Alternatively, parasites may select for hosts that minimize the fitness costs of parasitism they experience (virulence) without limiting within-host parasite growth. This reduction of the fitness costs of parasitism is often referred to as tolerance (Boots *et al*. 1999; Little *et al*. 2010). Potential hosts may combat the costs of parasitism with parasite avoidance, recovery, or tolerance strategies of resistance (Boots *et al*. 1999), where avoidance is acquiring a lower susceptibility to infection, recovery is overcoming infection, and tolerance is the process of decreasing pathogenicity (Boots *et al*. 1999).

Since hosts and parasites can select on each other, host-parasite systems provide some classic examples of coevolution (Woolhouse *et al*. 2002; Duffy & Forde 2009; Sadd *et al*. 2011). Understanding hosts' response to parasitism as well as the variation in fitness consequences of parasitism for hosts and parasites is important in order to better understand the coevolutionary process in general. The environment is a key player in determining the nature of coevolution. Environmental conditions, as well as host and parasite genotype, can determine host responses to parasite exposure, and different host genotypes are likely to succeed under different conditions (Lazzaro *et al.* 2008).

The trade-off hypothesis suggests that virulence is a direct result of parasite transmission (Alizon *et al.* 2009). This means that, to achieve maximum fitness, a parasite must be virulent enough to grow well within the host, but not so virulent as to kill the host too soon. Parasites steal large amounts of energy from the host that could be devoted to growth and reproduction, and different hosts are affected more or less severely (Vale *et al.* 2011). In previous studies, parasites that grew more quickly did not necessarily affect the host more negatively; for example, in other *Daphnia*-parasite studies, faster growing parasites did not reduce host fitness more than a slow growing parasite, indicating that virulence depends on a combination of factors (Little *et al.* 2008; Vale *et al.* 2011). Further, hosts with increased tolerance experienced more cases of parasitism. (Vale *et al.* 2011).

Food quality is an important factor influencing host-parasite interactions. It can impact both the host and the parasite, in part since nutritional deficiencies can limit metabolic activity (Frost *et al.* 2008). For example, in a snail-trematode system, when snails are exposed to poor quality resources, they were more likely to die from a parasitic infection (Krist et al. 2004). This indicates that hosts can combat infection more effectively when given higher quality resources. However, snail hosts reared in low quality environments were no more susceptible to infection by a trematode than hosts reared in high quality food (Krist et al. 2004). This is surprising since it would seem that parasites could more easily overtake a nutritionally deficient host. Another example of an effect of food quality on host-parasite interactions comes from a bumblebee-trypanosome system. In this system, bumblebees exhibit condition-dependent virulence when infected by an intestinal parasite (Brown *et al*. 2000). Poor resource conditions can cause a 50% increase in mortality rate for bumblebee hosts when they become infected with the trypanosome (Brown *et al.* 2000). Parasite fitness is also affected by the food quality of the host. In a *Daphnia*-parasite system, it was found that parasites were less likely to infect nutrient-deficient hosts; in addition, they were not as virulent once inside a nutrient deficient host (Frost *et al*. 2008).

I examined the influence of host food quality on parasitism using a *Daphnia*-parasite system. Previous studies have indicated that higher resource levels favor increased parasite replication within the host and early host death (Hall *et al.* 2009). The purpose of this study was to determine the effects of the food quality experienced by hosts on host and parasite fitness. We used *Daphnia dentifera* and its yeast parasite *Metschnikowia bicuspidata* to investigate whether the parasites suffer from a poor host environment or the hosts suffer from increased infection when provided with lower quality food. We were interested in investigating the effects of food quality because resource quality can often be a limiting factor for *Daphnia* populations in lakes (Hall *et al.* 2009).

It seems intuitive that hosts provided with higher quantities of food would be more likely to survive under parasitic infection since they would have more resources to invest in defense (Jokela *et al.* 2000). It would also seem that, in addition to higher food quantity, better food quality should enhance host fitness, since a more nutritious food would enable the host to mount more defenses against the parasite. However, when a host consumes higher food quality, the parasite inside the host may be able to benefit as well. For example, the parasite may be able to mount stronger attacks against the host, since they are in a better environment (as compared to a parasite in a poorly fed host), making it harder for the host to defend itself against the parasite. We predicted that the parasite would benefit more from a weakened host since the parasite does not have the same nutritional requirements as the host and a weakened host could not mount effective defenses. Hosts fed lower quality food were predicted to filter more food in order to acquire the necessary nutrients, thereby ingesting more spores and decreasing their fitness. However, we also predicted that parasite growth would be lower in low quality food environments, since the host might not receive enough nutrients.

METHODS AND MATERIALS

Study Organisms

Daphnia dentifera is a common planktonic crustacean that inhabits lakes in temperate North America (Duffy *et al*. 2011). This species is commonly infected by a number of parasites, including the parasite *Metschnikowia bicuspidata* (Duffy *et al.* 2010). *Daphnia dentifera* can reproduce either sexually or asexually, depending on the environmental conditions (Hebert 1995). When conditions are favorable, *D. dentifera* reproduce by amicitic parthenogenesis, which creates genetically identical female offspring (Carvalho 1994). Different genotypes have different relative fitness depending on their habitat (Hebert 1995). Their unusual reproductive biology means that *Daphnia dentifera* genotypes can be easily maintained as clonal lines in the laboratory.

Metschnikowia bicuspidata is a parasite that infects *D. dentifera* and reduces both the fecundity and lifespan of the individual it infects (Duffy *et al.* 2011). The *D. dentifera* become infected when they ingest spores orally as they consume their algal food (Hall *et al.* 2009). It is therefore possible to propagate *M. bicuspidata* by exposing mature spores to healthy hosts in the laboratory.

Two varieties of algal food were used: *Ankistrodesmus falcatus* and an *Oocystis sp.* strain collected by Hall et. al (2012) in the field so its species was unknown. *Ankistrodesmus* was the high quality food that *Daphnia* prefer. *Oocystis* was the low-quality, and thus less favorable, food for the *D. dentifera.* The presence of a protective sheath around *Oocystis* cells makes it more difficult for *Daphnia* to gain nutritional benefit. Both *Ankistrodesmus* and *Oocystis* are common to freshwater ecosystems; *Oocystis* is a lighter green than the *Ankistrodesmus*, (Hepperle *et al*. 2000). Cultures of *Oocystis* were prepared by adding the algae to media and incubating the culture under constant light for several days. The media was autoclaved to ensure sterility before the culture is added. After several days the cultures were growing visibly and samples were analyzed under a microscope to ensure that the correct species were growing in the chemostats. We standardized food to create equal amounts of carbon between treatments. All *Daphnia* were fed the same quantity of food, measured as mg of carbon (C), but treatments differed in food quality, i.e. how easily the *Daphnia* can digest and assimilate the carbon. We standardized by C content by collecting both spectrophotometer and dry mass readings for a variety of concentrations of both *Oocystis* and *Ankistrodesmus* and performing a regression between these readings for each of the algal species.

Experimental procedure

This study examined whether parasite fitness depends on the quality of the environment provided by the host. Specifically, it tested whether: the quality of food consumed by the host before and/or after exposure to the parasite affects (1) the probability of infection given exposure to the parasite; (2) parasite fitness (measured as the number of mature transmission spores on day of host death); and (3) the relationship between host fitness (measured as host survival and offspring production) and parasite fitness (measured as spore production). To prepare the experimental animals, 50 adult *Daphnia* (8-10 day old) from the same clonal lineage, H37 (Auld *et al.* 2012**),** were isolated and fed 2 mL of

Ankistrodesmus. After a period of 24 hours, about 200 juveniles were produced parthogenetically and were harvested. Each individual was put into a 100 mL beaker, and fed either high or low quality food. There were four treatment groups: High quality food as a juvenile and adult ("HH"), high quality food as a juvenile and low quality food as an adult ("HL"), low quality food as a juvenile and high quality food as an adult ("LH"), and low quality food as a juvenile and low quality food as an adult ("LL"). Each treatment had 33 individuals.

A culture of the yeast parasite *M. bicuspidata* was created by isolating juvenile *D. dentifera* and infecting them with a set number of spores per beaker. Parasite exposure occured on day 7, between the juvenile and adult stages. Each replicate *Daphnia* was exposed to 1000 *M. bicuspidata* spores per mL in 50mL of culture medium. All replicates then received low amounts of high quality food, *Ankistrodesmus* (50% of the standard amount) for 24 hours in order to promote grazing on the parasite. Proportion of animals infected, host mortality and number of offspring per host was recorded to assess host fitness. Parasite fitness was measured by crushing up each dead host and counting the number of spores inside each individual.

Analysis

The proportion of infected hosts was analyzed using a generalized linear model with binomial errors; food treatment was included as a fixed effect. The generalized linear model was also used to determine offspring number in infected hosts as well as spore counts with Poisson errors. Finally, a Cox's proportional hazards analysis was used to determine survival.

RESULTS

Food quality significantly affected infections; a greater proportion of individuals fed high quality food as juveniles became infected (contrast of HH and HL treatments vs. LH and LL treatments, $\chi^2 = 10.13$, DF = 3, $p < 0.05$: Figure 1). However, there was no significant relationship between spore production and food quality treatment in infected individuals (Figure 2: $\chi^2 = 1.75\times10^6$, DF = 3, $p = 0.22$). There was a positive relationship between parasite spore production and host fecundity (Figure 3: $\chi^2 = 3.87 \times10^6$, DF = 1, $p < 0.0001$), but this did not depend on food treatment (food treatment: $\chi^2 = 4.19 \times10^5$, DF = 3, $p = 0.64$; host offspring × food treatment: $\chi^2 = 1.47 \times10^5$, DF = 3, $p = 0.90$). Finally, there was no significant difference in the survival of individuals between food treatments (Figure 4: $\chi^2 = 4.48$, DF = 3, p = 0.21).

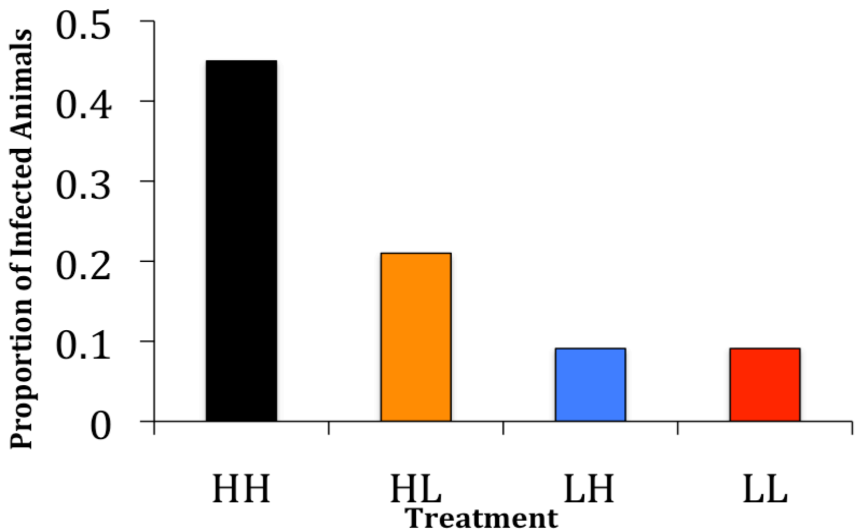

Figure 1. Effects of food quality on infection. More animals fed high quality food as juveniles were infected. When host fed high quality food as a juvenile and adult, it can invest more energy in defense against parasites. HH= High quality food as a juvenile and adult, HL= high quality food as a juvenile and low quality food as an adult, LH= low quality food as a juvenile and high quality food as an adult, LL= low quality food as a juvenile and low quality food as an adult.

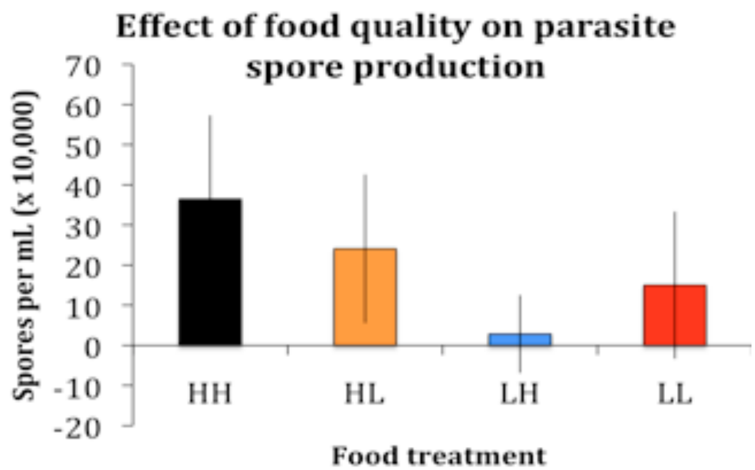

Figure 2. There was no overall effect of food quality treatment on spore production in infected individuals. HH= High quality food as a juvenile and adult, HL= high quality food as a juvenile and low quality food as an adult, LH= low quality food as a juvenile and high quality food as an adult, LL= low quality food as a juvenile and low quality food as an adult.

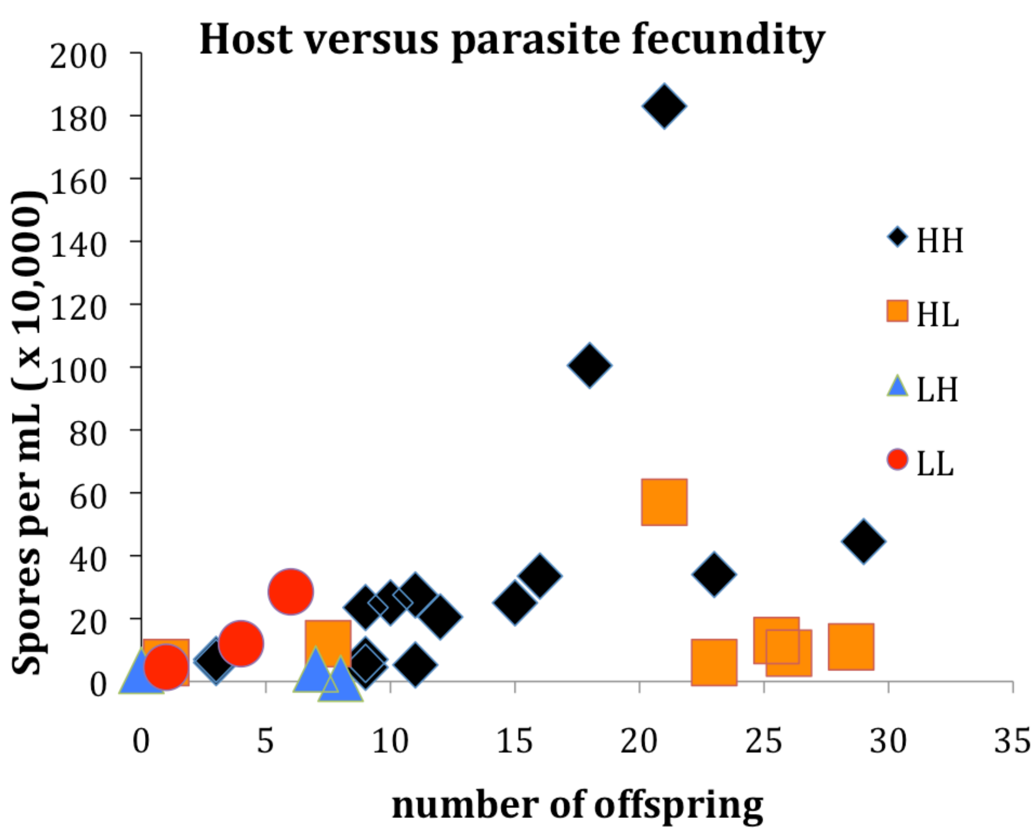

Figure 3. The relationship between number of offspring and spores per host is not affected by food treatment. Higher food quality increases likelihood of infection but parasitized females fed higher quality food have more offspring. There is a positive relationship between number of host offspring and number of parasite spores. HH= High quality food as a juvenile and adult, HL= high quality food as a juvenile and low quality food as an adult, LH= low quality food as a juvenile and high quality food as an adult, LL= low quality food as a juvenile and low quality food as an adult.

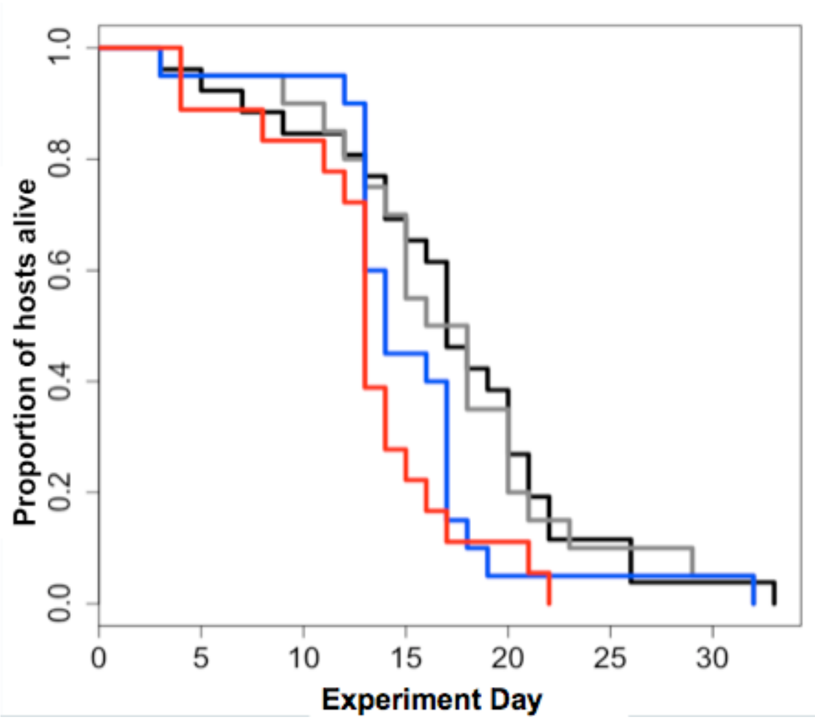

Figure 4. Survival plot (day of death) for infected individuals in each of the four treatments: HH= black, HL= grey, LH=blue, LL=red. There was no significant difference between the day of death of infected individuals between treatments. HH= High quality food as a juvenile and adult, HL= high quality food as a juvenile and low quality food as an adult, LH= low quality food as a juvenile and high quality food as an adult, LL= low quality food as a juvenile and low quality food as an adult.

DISCUSSION

Of the four treatments, the highest proportion of infected individuals came from the high quality for life treatment group, indicating that food quality influences the likelihood of the parasite penetrating host tissues and establishing an infection. This indicates that an individual fed high quality food is more susceptible to disease, not more resistant to parasitic attack. There was a positive relationship between parasite spore production and host fecundity, but this did not depend on food treatment. These results indicate that more infected individuals also produced more offspring, meaning that both parasite and host seemed to benefit from the interaction: the parasite was more successful in hosts, and the hosts reproduced more, increasing their biological fitness.

These results are consistent with other studies of the effects of food quantity and quality on host-parasite interactions. Bittner *et al.* (2002) examined a host-parasite system of the host *Daphnia galeata* and the parasite *Caullerya mesnili* (a protist parasite) and manipulated food quantity of the host (Lohr *et. at.* 2010). They found that infected individuals had lower fecundity regardless of food quantity, which is interesting because the results of our experiment indicated that higher food quality increased fecundity (Bittner *et al.* 2002). Perhaps only quality of food has effects on fecundity. However, more *C. mesnili* spores were produced in hosts fed high quality food, indicating that parasites have higher growth rates in well-fed hosts (Bittner *et al.,* 2002). These data is consistent with our findings that more hosts fed high quality food were infected.

Another study using the *Daphnia dentifera-Metschnikowia* system found that *Daphnia* populations decreased as algal resource quality improved (Hall *et al.,* 2009). Hall *et al.* (2009) attribute this relationship to seasonal changes in temperature as well as parasites becoming more virulent in hosts consuming high quality food. It was also found that fecundity and spore production increased with high quality food, which is consistent with our results that high food quality is beneficial to both host and parasite. High food quality enables the host to produce more offspring, putting new uninfected and healthy individuals into the lake ecosystem; high food quality also allows the parasite to produce more spores within each host (Hall *et al.,* 2009). Hall *et al.* (2009) refer to this mutually beneficial relationship an energy-theft mechanism because hosts using high quality food have more energy to expend and more energy and nutrients are made available to parasites to steal from within the host.

In this study, food quality had no significant effect on the number of spores produced in infected individuals, indicating that once the parasite infiltrated the host, it could replicate successfully within both hosts fed poor quality food and well fed hosts. However, higher proportions of well-fed *Daphnia* became infected, indicating that there could be some sort of benefit for the parasite to be in an environment with well-fed hosts. We found a significant relationship between reproduction by infected hosts and spores produced per infected host. Several explanations could be offered for this positive relationship. For example, well-fed hosts might have higher filtration rates and ingest more spores while eating, thus encountering more parasite spores, leading to the observed proportion of well fed infected individuals. Also, hosts fed high quality food could have more resources to use and the nutrients from the food could be used by both the host for reproduction and by the parasite for creating spores. Alternatively, the host could be increasing its fitness level by producing more offspring before death once infected as an adaptation to disease. Future studies should be conducted to deduce the drivers of this phenomenon.

Earlier studies using alternate model organisms have found that food quality affects host-parasite interactions. Krist *et al.* (2004) used a snail-trematode system to find that animals exposed to poor quality resources were more likely to die from a parasitic infection. Snail hosts reared in low quality environments were no more susceptible to infection by a trematode than hosts reared in high quality food, indicating that hosts and parasites both use food quality as a means to increase personal fitness (Krist *et al.* 2004). This indicates that host-parasite interactions are variable across organisms, especially in relation to food. Some studies have found that the parasite has higher growth rates in well-fed hosts (Bittner *et al.,* 2002), while our study found that there was no significant difference in spore production with survival or food treatment. To conclude, food quality seems to be an important player in a wider range of host-parasite systems, but the effects are variable.

Food quality varies considerably in natural systems, and this can have profound effects on interactions between hosts and their parasites. However, the relationship between food quality and parasitism is clearly complex: increased food quality can favor the host, the parasite or indeed both parties (as shown in this study), depending on the host-parasite system. Developing a better understanding of how food quality affects parasitism is, nevertheless, an important stage in understanding the evolution and ecology of infectious disease.

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